

# Untangling Terpenes

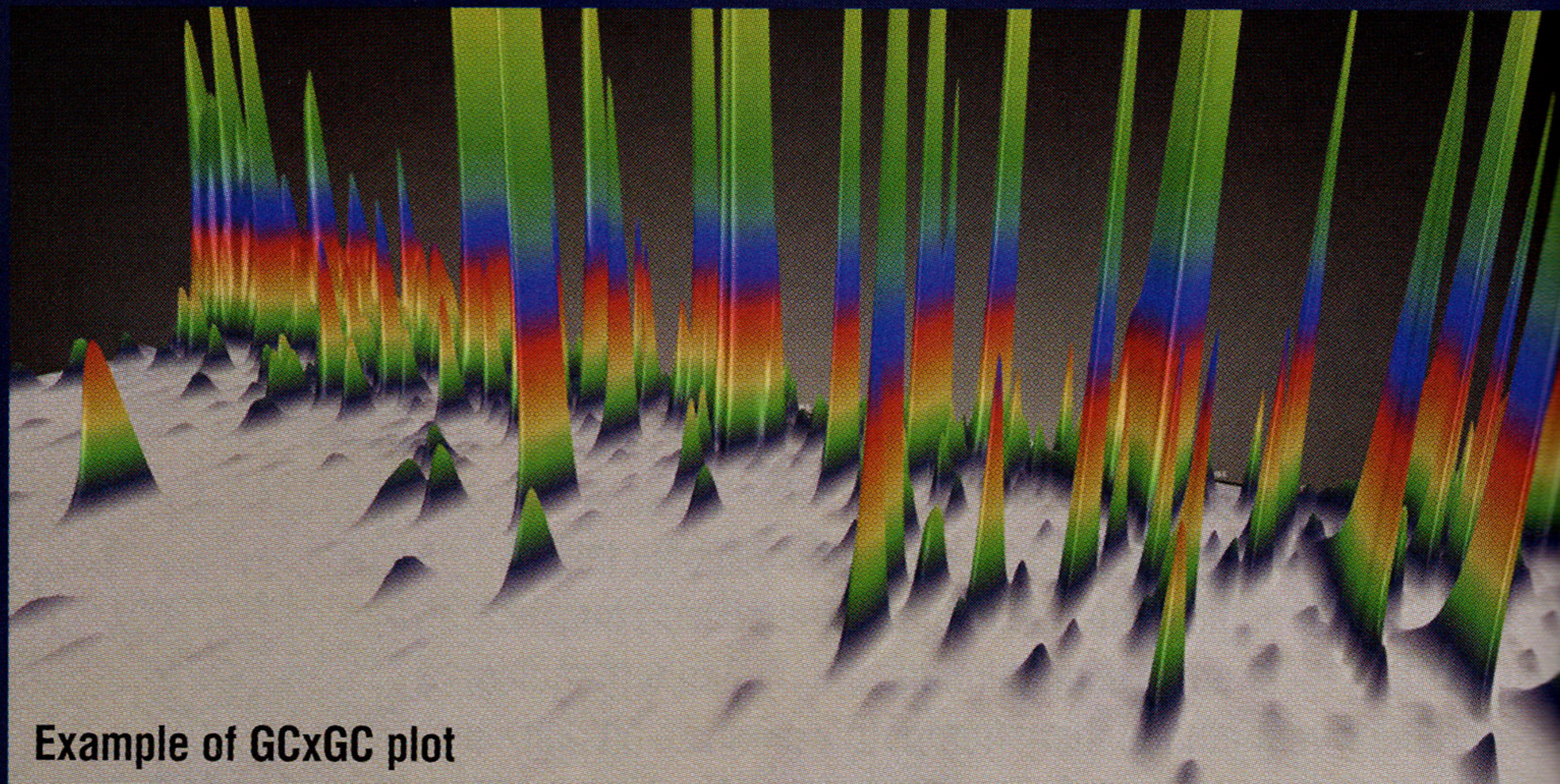
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While the domino-effect of recreational legalization is a welcome development for everyone involved in the commercial cannabis industry, past years of criminalization have prevented a great deal of viable research. Now, held up to the light of public scrutiny and the gauntlet of verifiable, repeatable testing, cannabis is revealing itself as increasingly complex. While scientific advances in the field are occurring, particularly regarding cannabinoids, the full nature of terpenes remains largely unexplored.

Ultimately, a deeper understanding of these compounds is necessary to answer crucial, but industry-specific questions. For example, what are agreeable definitions for terms like 'tangie', 'skunk', and 'diesel' in regard to the flavor and terpene content of particular cultivars? Granted, subjects like these may seem esoteric or unimportant to people unfamiliar with cannabis. However, they are integral to the chemists, analysts, manufacturers, patients, and recreational customers seeking a particular flavor, sensorial experience, or effect in cannabis and/or terpene-related products.

## Legwork

Abstrax' main focal point is the research behind these mysteries. So, it's our goal to continually push our own intellectual limitations regarding theoretical and analytical terpene testing. This pursuit means continually seeking improved equipment, updating operating protocols, and placing more



Example of GCxGC plot

intense scrutiny on the variable details of industry-related developments.

As terpenes are radically affected by a wide range of factors, even small changes in a growing cycle, transportation method, storage methodology, and/or mixing procedure can be drastic. They're easily damaged, degrading through exposure to sunlight, oxygen, and even the unavoidable passage of time. When this happens, a terpenoid is often formed. Over 100 terpenes have been identified in various cannabis samples, each with unique characteristics like taste and effect that are vulnerable to this degradation. In addition to the other factors mentioned, the methods by which terpenes are studied must be carefully selected to unlock their mysteries.

## Processing and Separation

The cannabis industry is still pretty new, so there's few established standardized procedures for analytical testing. These methods will usually entail some type

of chromatography which separates molecules based on their polarity. The laboratory process for the separation of a mixture, chromatography consists of a liquid or gas (called the mobile phase) moving a mixed sample through a solid structure (the stationary phase). This usually happens within a column, a sealed, metal cylinder. In other words, sample material is forced through a tube full of filters so a detector can measure its individual parts. Here are two analytical methods common in cannabis testing labs:

### 1. High-Performance Liquid

**Chromatography (HPLC):** Inert liquids are pumped through a short column at a specific pressure. The flow is standardized so that a sample can be injected into the otherwise stable system. As the material moves through the column, filtration processes separate the various parts of the sample. This data is measured by built-in detectors and sent to a computer.

**2 Gas Chromatography (GC):** An inert, carrier gas is released into tubes moving through a column within a specialized oven. This flow is sustained while a sample is introduced to the system via an injector. These small amounts pass through the filters of the column, which divide the material. Separated analytes are measured using a detector, such as a flame ionization detector (FID), which combusts the separated compounds, thereby producing ions.

### The Right Tool for the Job

Like their names suggest, the primary difference between the two is the use of liquids relative to gases for separation. Either way, inert liquids or gases force a given sample through a separation medium, dividing that formerly mixed material into individual, measurable components. Also, GC is typically used for volatile species, like terpenes or residual solvents, whereas LC is often selected for the separation of non-volatile, or thermally labile compounds, such as distinguishing THCA and THC.

Another significant difference between the two is that liquids are more viscous than gases. This means that HPLC requires a significant amount of pressure to move those liquids through its column. That additional pressure necessitates a thicker, stronger column to withstand the pumping machinery. While this process is ideal for samples with higher molecular weights, the additional and beefier equipment required is expensive, slow to implement, and can require lengthier training.

GC, on the other hand, doesn't require pressure pumps, so the more delicate machinery can be utilized. GC has the option to use capillary columns which require less material. Gaseous samples interact with the functionalized walls of the column, compared to the HPLC column, which is filled with tightly

packed material, often silica beads with octadecyl carbon sidechains attached. GC requires less sophisticated machinery and advanced training, and offers faster analyses than HPLC, again provided that the analytes are suitable for the method.

### Doubling Down

These analytical methods are widely used and accepted in modern pharmaceutical and medicinal fields. Terpenes are so profoundly intricate, however, that even the traditional chromatographic methods aren't always up to the task. Terpenes sometimes appear as isomers: compounds with the same elemental formulas, but with different molecular structures. Some detectors may be fooled when isomeric terpenes appear within a sample. Isomers often act differently than their counterparts, so there can be different physiological effects within the scope of a single terpene. This information further complicates the academic study of the 'entourage effect'. That is, the process through which terpenes interact with each other

and/or cannabinoids in various ways, depending on the mixture.

To get a better grasp on these topics, Abstrax utilizes Two-Dimensional Gas Chromatography (GCxGC). The process begins the same as GC - a sample is introduced to a column via injector. But, instead of passing through a detector at the end of that column, the material moves on to a second column with different selectivity before appearing in colored plot charts. This differentiation helps show the versatility within samples. One good example is that even though regular GC can distinguish some monoterpenes (10 carbon atoms) from sesquiterpenes (15 carbon atoms), GCxGC can distinguish in high resolution and look behind peaks that would otherwise be the sum of multiple peaks in a regular GC. Identifying the exact constituents of samples is integral to achieving better-structured characterizations of the various chemovars. The implementation of GCxGC has been massively beneficial in our efforts to improve our process and understanding.

